



TITLE:

Conformational Effect (Induced-Fit) on Catalytic Activity of α - Chymotrypsin (BIOORGANIC CHEMISTRY-Bioorganic Reaction Theory)

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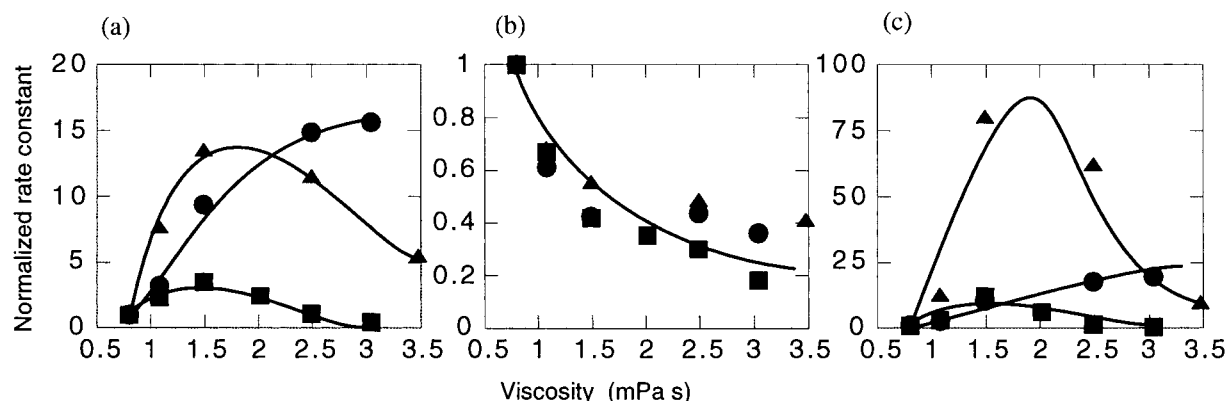
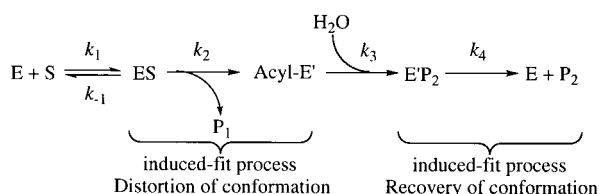


Figure 1. Dependence of kinetic parameters on medium viscosity: **1**, **2**, **3**: (a) k_{cat} , (b) k_2 , (c) k_3 .

i.e., a conformation that was not adopted to accommodate the acyl group, tends to eject the acyl group from its pocket so as to stabilize itself as the free enzyme. Thus, the decrease in k_2 is coupled by an increase in k_3 . However, the increase in k_3 with the increase in medium viscosity is not monotonic. Instead, it begins to decrease at about $\eta \approx 1.6$ mPa s. This is accounted for by the presence of an additional process in this step, which becomes unfavorable as the medium becomes more viscous. We assign this additional process to the product releasing step with a rate constant of k_4 . This assignment proposes that the conformation suitable for the acyl enzyme is also suitable for releasing P_2 , the acid part of the substrate. In other words, the release of free acid from the enzyme of undistorted conformation is not a simultaneous process. The release of P_2 requires energy corresponding to the release of distortion energy that exists in the conformationally distorted enzyme, and Scheme 1 is now revised to Scheme 2 by taking into account the induced-fit conformational adjustment for the release of P_2 .



Scheme 2

The shapes of the curves for k_{cat} shown in Fig. 1(a) are similar to those for k_3/k_4 shown in Fig. 1(c), which stems from the fact that this step is more sensitive process to the effect of induced-fit conformational adjustment than the k_2 step and the chemical (catalytic) reactivity of the enzyme is regulated by this process. It has been known that the rate-determining step in the enzymatic hydrolysis of an ester is the step in which the acyl enzyme is hydrolyzed into a free enzyme and an acid. Absolute values for k_2 and k_3 , as well as substrate

independence of the k_2 step shown in Fig. 1(b), also support the idea that the hydrolysis of acyl enzyme is (at least in part) the rate-determining step of the present reaction under normal conditions. However, interestingly, the rate-determining step is shifted to the k_2 step in the reactions occurring in highly viscous media for certain substrates. An exception is the reaction with **1**, where the rate-determining step is always in the k_3/k_4 step even in the most viscous medium studied.

The observation that the maximum position in the plot of k_3/k_4 step for **1**, if any, shifts to higher viscosity than the other amino acid substrates indicates that the reaction of this substrate is less sensitive to induced-fit conformational adjustment than the reactions of other substrates. The result obtained herein has good agreement with that obtained by proton inventory kinetics [3], where **1** was found to be associated by the movement of one proton whereas other amino acid substrates were associated with the movement of two protons at their respective transition states. In other words, **1** is not an appropriate substrate for studying the catalytic activity of α -chymotrypsin. Although, conventionally, **1** has been employed as a typical substrate in the study of catalytic activity of α -chymotrypsin, one should be very careful in extending the results obtained with **1** as a substrate to the discussion of the general mechanism of α -chymotrypsin hydrolysis.

Consequently, we now believe that both acylation/deacylation and release of products P_1/P_2 are dependent on the induced-fit conformational adjustment in the hydrolysis of an ester by α -chymotrypsin. More results should be accumulated using substrates of various types before we reach a conclusion.

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